PROPAGATION, GROWTH AND HAEMATOLOGY OF FISH CULTURED IN WATER FROM A NATURAL STREAM AND UNDERGROUND TUBEWELL SOURCES

ABSTRACT

Propagation, growth and haematology of *Clarias gariepinus* bred and reared in water from a natural stream at 5^o28'N, 7^o32'E (Nature stream) and two wells at 5.481^0N, 7.5370E (well-1) and 5.477^0N, 7.5390E (well-2) were studied. The study was carried out in search of suitable water within close proximity for fish propagation. Growth was evaluated once in two weeks. Hematological parameters (**such what????)** of the fingerlings were examined at 70 day post hatch. There was significant difference ($P<0.05$) in egg fertilization, hatchability, larval survival, growth and blood parameters among the treatments. Fish exposed to water from the natural stream **established….**impacted best propagation indices including fastest growth and highest Values of Packed Cell Volume **(PCV)** and Mean Corpuscular Haemoglobin Concentration(**MCHC)** of fish for well-1and well-2 were not statistically different ($P>0.05$). Apart from these two parameters, values of all the other blood parameters including growth were significantly lower ($P<0.05$) in fish exposed to water from well-2 than those from well-1. The disparities observed signifies differences in water quality which is indicative **for the** presence of contaminant not identified in this study. The report recommended inclusion of heavy metals and organic mineral analysis in future study to disband**….**identify the cause of the observed alterations.

Key words: Blood indices, *Clarias gariepinus*, Fish propagation, Growth, Surface water,

Underground water and Water quality

INTRODUCTION

Fishes being poikilothermic are very sensitive to changes in their **ambient aquatic** environment (Uka, 2021). Fazio *et. al.,* 2013) clearly stated that fish live in very **friendly…**intimate contact with their environment, and are therefore very susceptible to physical and chemical changes which may be reflected in their blood components. Borkovic *et al.*, (2008) noted that fish are largely used for the assessment of aquatic environment quality and are **considered a** bio-indicator **for the** environmental pollution. Zeitoun and *Mehana (2014)* **stated** that studies on fish can play significant role in assessing potential risk associated with contamination of aquatic environment.   Fernades and Mazon (2003) had earlier reported that fish hematological changes **are affected** with **its surroundings.** They noted that evaluation of **blood parameters** helps to understand the influence of environment on fish **welfare.** Therefore, analysis of haematological parameters is a diagnostic technique to understand health of fish in any environment. De-Pedro *et al.,* (2005) re-echoed that monitoring fish health can be done using haematological and biochemical profile of its blood. Satheeshkumar *et al*., (2011) observed that haematological and biochemical changes in blood are important indicators used in monitoring **both** physiological and pathological **alterations** in fish. They concluded that qualitative and quantitative variations in haematological parameters including the red blood corpuscles (RBC) and white blood corpuscles (WBC) numbers, cell proportions of leukocyte, the amount of haemoglobin and the

size of WBC and RBC are the most significant findings as regards diagnosis. Bahmani *et al*., (2001) reported that analysis of haematological and biochemical indices in the blood of farmed fish is good for identifying the health status of farmed fish as they provide reliable information on metabolic disorders and deficiencies. Therefore, haematology has been used as an index of fish health status in a number of fish species to detect physiological changes, as a result of exposure to different stressful condition such as handling, pollutants, metals, hypoxia, anaestehtics and acclimation (Alwan *et al*., 2009). Yaribeygi *et. al*., (2017) reported that certain environmental stressors can alter blood characteristics with resultant disruptions in metabolic activities leading to reduced growth rate and impairment of reproductive process,

Fazio *et. al*., (2013) showed that data on haematological parameters are considered a good indicators for health status of fish in response to changes related to nutrition, water quality as well as disease. The health status is reflected on growth and reproduction (London and Volkoff, 2019). Gorjipour (2014) noted that the most common sources of water for aquaculture are wells, springs, rivers and lakes. Wells and springs are considered the best sources of water used for aquaculture. Rivers and other open water bodies have variable chemical and physical characteristics that make them unpredictable in terms of reliability for aquaculture purpose. The present study compared between the growth and haematological characteristics of *Clarias gariepinus* bred and reared in water from different sources.

MATERIALS AND METHODS

Sources of experimental waters: Sources of the experimented waters were from a natural stream at 5^o28'N, 7^o32'E (Nature stream) and two neighborhood wells at 5.481^0N,7.537^0E (well-1) and 5.477^0N,7.539^0E (well-2) .

Propagation and rearing: Sexual products (what ???)…(eggs and sperm) were obtained by artificial propagation. Sexually matured female *C. gariepinus* were selected on the basis of ovarian biopsy while males were chosen based on pointed urogenital papillae. The papillae of the selected males were hyperemic at the tips…tip. Hormone administration was as implemented by Uka and Obijiaku (2019). Eggs and sperm were procured as described in Uka and Obijiaku (2019). A total of four females and two males were used. The eggs from the four females were pooled together and were fertilized with pooled sperm from the two males. The fertilized eggs were incubated uniformly in three large concrete or glass or plastic????....congrete hatching tanks each containing water from any of the 3 different sources (Nature stream, Well-1 and Well-2). Hatchlings were monitored in these tanks for 10 days post hatch. Twenty liters of water for each treatment was got…collected with measuring cylinder into 25 litres capacity bowls for larval rearing. The larval rearing in each treatment was conducted in quadruplet. Forty healthy fry from each nursing tank were distributed in equal number into their respective treatment water in the bowls. The fish were nursed in these bowls for 60 days. All through the rearing period, the fish were fed to satiation with 42% crude protein commercial diet (Sotolu, 2010).

Growth monitoring: Growth was monitored by measuring the weight and length of the fish on fortnight basis. The instruments used for growth monitoring were metre rule, pair of divider and sensitive weighing balance (Mettler Toledo, PL 303). Growth parameters determined were Daily

specific growth rate (DSGR), established from the formula: DSGR $(\%) = \frac{\log W2 - \log W1}{T - t} x\ 100$, mean growth rate (MGR): MGR$=\frac{W2 - W1}{0.5(W2 - W1)t} X100$ and percentage weight gain %WG$=\frac{Wt - Wo}{Wo} x100$. Where W2 = final weight, W1= initial weight, T= final time and t= initial time.

Analysis of blood parameters: Blood samples were collected after 70 days post hatch from tail vein with insulin syringe. The samples were transferred to heparinized tubes and taken to laboratory for analysis. The tested haematological parameters were packed cell volume (PCV), Red blood cell (RBC), Haemoglobin (Hgb), white blood cell (WBC), mean cell volume (MCV), mean cell hemoglobin (MCH) and mean cell hemoglobin concentration (MCHC). Packed cell volume (PCV) was estimated after placing sealed microhaematocrit tube in a centrifuge at 10:500 rpm using micro haematocrit and the PCV was read off. Red cell count (RBC) was determined from a small amount of blood diluted 1 in 200ml with a solution of lithium heparin anticoagulant, to preserve the corpuscles. The corpuscles were then counted in a special counting chamber (Neubauer's slide chamber) with the use of microscope. White blood cells (WBC) were counted by Neubauer's improved haemocytometer using Hyem's and Turks solution as a diluting fluid. Haemoglobin was estimated using SAHLI'S method (Acid Hematin Method). Blood was added to N/10 Hydrochloric acid (HCl). Colour change was matched with the standard reference Sahli's apparatus to read concentration of haemoglobin in the blood. Mean corpuscular volume (MCV) was calculated from the volume of red cells and number of red cells in 100ml of blood and expressed in fento litre (FL). MCV was mathematically estimated from the formula: $\frac{Vol.of\ red\ cells\ in\ 100ml\ blood}{Number\ of\ red\ cells\ in\ 100ml\ blood} = M.C.V$. Mean corpuscular haemoglobin (MCH) was calculated by dividing the amount of Hgb in 100ml blood with the number of red cells in the same volume of blood and expressed in pictogram. It was mathematically estimated with the formula: M.C.H$=\frac{Haemoglobin\ in\ grams\ per\ 100ml\ blood}{Number\ of\ red\ cells\ per\ 100ml\ blood}$. Mean corpuscular haemoglobin concentration (MCHC) was calculated from haemoglobin in grammes and the volume of packed cells in 100ml. It was mathematically estimated with the formula: M.C.H.C$=\frac{Haemoglobin\ in\ grams\ per\ 100\ mlblood}{Volume\ of\ red\ cells\ in\ ml\ per\ 100\ ml\ blood}$ x100g per decilitre $=\frac{Haemoglobin\ content}{Haematocrit}$ x 100g/ dl. (Saravanan *et. al.,* 2011).

Water quality monitoring
The water quality parameters in the egg incubator and in larval rearing tanks were monitored during the study. Dissolved oxygen was monitored with Dissolved Oxygen meter (EXTECH SDL 150). Temperature and pH were monitored with Hanna Instrument pH metre (HI 991350) (Bhatnagar and Devi,2013).

Data analysis
The data obtained were analyzed using SPSS 15.1. One-way ANOVA was employed. Where difference was found, Duncan Multiple Range Test was used to separate the means. Presence or absence of statistical difference was determined at 5% probability level.

RESULTS AND DISCUSSION

<center>**Results**</center>

Propagation

Percentage <mark>of</mark> egg fertilization in waters from the three sources (nature stream, Well-1 and Well-2) declined from 90.72 % through 79.66 % to 57.50 % respectively (Table 1). The difference in the fertilization rate was significant ($P<0.05$). Egg hatching success (78.0 %, 74.68 % and 49.65 %) and larval survival (86.8 %, 78.7 % and 55.5 %) in the same order were equally significantly different ($P<0.05$) among the treatments (Table 1).

Table (1: Effect of water from different sources on hatchery propagation of *C. gariepinus*

Parameters	Natural Stream	Well-1	Well-2	Statistics
Egg fertilization (%)	90.7	79.7	57.5	*
Egg hatching (%)	78.0	74.7	49.7	*
Larval survival (%)	86.8	78.7	55.5	*

*=Significant at 5% level

Growth

Growth was significantly different among the treatments ($P<0.05$). Final weight attained declined from 26.54±13.27g through 19.27±7.61g to 17.71±6.27g in Nature stream, Well-1 and Well-2 respectively. Mean weight gain of 3.69±2.53g, 2.01±1.39g and 1.45±1.13g were achieved in the same order while Daily specific growth rate was 0.18±0.20, 0.22±0.16 and 0.16±0.16 respectively (Table 2).

Table 2: Growth performance of *C. gariepinus* in Water from three different Sources

Parameters	Natural Stream (mean ±SD)	Well-1 (mean ±SD)	Well-2 (mean ±SD)	Statistics
Final Mean wt (g)	26.54±13.27[a]	19.27±7.61[b]	17.71±6.27[c]	*
Mean wt gain (g)	3.69 ±2.53[a]	2.01±1.39[b]	1.45±1.13[b]	*
DSGR	0.18±0.20[a]	0.22±0.16[b]	0.16±0.16[b]	*

DSGR = Daily Specific Growth rate, *=Significant at 5% probability level ($P<0.05$). SD= Standard deviation. Values in the same column with different superscript letter are significantly different.

Haematology

The fish reared in natural stream showed highest values in all the blood parameters studied. Values of PVC and HCMC were not significantly different ($P>0.05$) in water from well-1and well-2. However, their values in well-1 were higher than in well-2. Apart from these two parameters, values of all the other blood parameters were significantly lower in fish exposed to well-2 than those exposed to well-1 (Table 3).

Table 3: Hematological profile of *clarias gariepinus* cultured in water from 3 different sources

Treatments	Blood parameters						
	RBC	WBC	PCV	HG	MCV	MCH	MCHC
Natural	2.5933	3.7900	28.3333	5.9667	38.3400	14.2100	18.3133

stream	±0.14844^a	±0.16093^a	±2.51661^a	±0.20817^a	±0.93664^a	±0.63695^a	±1.04242^a
Well-1	1.6233	2.6733	22.3333	4.7000	29.9933	12.0933	15.9467
	±0.18175^b	±0.21385^b	±1.52753^b	±0.10000^b	±0.75639^b	±0.29687^b	±0.40612^b
Well-2	1.3400	1.9800	20.0000	3.5667	29.8600	11.3900	15.3567
	±0.08888^c	±0.06000^c	±1.73205^b	±0.25166^c	±1.27389^b	±0.30265^c	±0.39273^b
Statistics	*	*	*	*	*	*	*

*=Significant at 5% probability level (P<0.05). Values in the same row with different superscript letter are significantly different. Blood parameter values = mean ± standard deviation

Water quality

Evaluation of the physicochemical parameters of the **tested** waters showed a satisfactory quality for hatching and rearing of *C. gariepinus*. Temperature was not significantly different among the treatments (P>0.05). The mean value of temperature in the incubation and the rearing tanks ranged between 28.00±0.16 and 28.80±0.18ᵒC. The mean pH value ranged from 6.06±0.49 to 6.54±0.87, while the dissolved oxygen ranged from 4.23±0.59 to 6.71±0.08mg/l. There was significant difference (P<0.05) among the treatments in dissolved oxygen concentration. Total dissolved oxygen in Well-2 (4.23±0.59) was significantly (P<0.05) lower than the total dissolved oxygen in Well-1 (6.06±0.49) and nature stream (6.71±0.08mg/l)-Table 4.

Table 4: Water Quality

Treatments	pH	DO(mg/l)	Temperature(^{o}C)
Nature Stream	6.54±0.87	6.71±0.48^a	28.80±0.18
Well-1	6.06±0.49	6.34±1.11^a	28.00±0.166
Well-2	6.34±0.38	4.23±0.59^b	28.50^a±0.20
Statistics	ns	*	ns

*=significant at 5% probability, ns=not significant. Parameters reported as mean ± standard deviation

Discussion

Disparities observed in egg fertilization, hatching and larval survival could **release….**be from the significant differences observed in level of dissolved oxygen among the treatments. Uka and Kalu (2019) reported increased egg hatching and larval survival with increase in levels of dissolved oxygen. They further reported that fluctuation in hardness enhanced egg hatching and suggested that fluctuation in hardness could be induced to enhance egg hatching during hatchery operations.

The observed differences in growth were evidence in the chemical, physical and biological state of the waters. Makori *et. al.,* (2017) reported increase in growth rate of tilapia as temperature and DO increased within optimal limits.

Mannan *et. al.,* (2012), revealed that water quality parameters, stocking density and species composition influenced the growth and production of fish in semi-intensively managed aquaculture farm. Imsland *et al.,* (2007) and Bhatnagar and Devi, (2013) recognised the

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importance of temperature and dissolved oxygen respectively in the growth of fish. Optimal water quality is therefore essential for fish growth. Fish growth is generally expected better in water of optimal levels of DO and temperature among other parameters.

The disparity in the blood parameters of the fish reared in different waters signifies differences in the quality of the waters. Fernades and Mazon (2003) reported that fish blood changes with a change in the environment where it lives. Fazio *et. al.,* (2012) clearly showed that the environment in which fish live and the conditions governing them influence the metabolite content in their blood. Significantly higher values of all the blood parameters and faster growth rate in fish reared in water from the natural stream implies superiority of the water to the tube-well sources. Fernades and Mazon (2003) further reported that evaluation of haematology helps to understand the influence of environment on fish wellbeing. Therefore, analysis of haematological parameters as a diagnostic technique is appropriate in seeking information on the health of fish cultured in different waters as carried out in this study. The decreased value of RBC recorded in well-2 implies low haemoglobin with resultant low oxygen carriage and poor carbon dioxide excretion. These could be responsible for the poor growth observed in well-2. The poor production of RBC and Hgb which is diagnosed in anaemic malady may be evidence of water quality deterioration as a result of presence of contaminant(s) not identified in this study. Zhang *et. al*., (2020) reported that presence of benzene derivatives can cause low RBC and low Hgb production in human bone marrow cells. This could also happen to fish in water contaminated with benzene derivatives. Fazio *et. al*, (2012), reported that the presence of any substance in water produces changes in their quality which are not always favourable to development and survival of aquatic organisms. Mean Cell Haemoglobin Concentration (MCHC) and the values of Packed Cell Volume (PCV) and Mean Cell Volume (MCV) were low either because the cells are small or because the haemoglobin concentration is low. Ishikawa *et. al*, (2007) stated that when the water quality is affected by toxicant, any physiological change will be reflected in the values of one or more of the biochemical and haematological parameters. Saravanan *et. al.,* (2011) showed that in fish exposed to different agents, the MCHC and MCH values decreased with respect to control group. Low concentration of MCHC in well-1 and well-2 is due to decrease in Hgb that could be attributed to its decreased synthesis or its oxidation into methemoglobin. Kroupovaa *et. al*., (2008) noted that major outcome of nitrite poisoning is the oxidation of haemoglobin to methemoglobin in erythrocytes. On the other hand, Sahiti *et. al.,(*2018) reported impact of water quality on haematological and biochemical parameters of fish indicative of stress caused by water pollution with heavy metals. It is therefore recommended that a more detailed investigation should be carried out to ascertain the contaminant(s) in the waters that are responsible for the low values of these blood parameters in inhabiting fish. This is crucial in any attempt to purify available water for food fish propagation. Therefore, this report recommends expanded analysis of the waters to include heavy metals and organic mineral parameters in future study to unravel the cause of the observed differences.

Conclusion

Fishes are reported to be sensitive to changes in their aqueous environment. These changes may be reflected in their blood, growth and reproductive parameters. As a result of this, fishes are used as bio indicators of aquatic pollution. This study reported fish growth, haematology, egg hatching and larval development in water from different sources. The study was conducted in search of suitable water within close proximity for fish seed propagation. The findings showed disparities in the impacts of the waters on the parameters studied. It provided a guide in choice of water for fish propagation in the study area. However, it recommended a more detailed investigation to ascertain the contaminant(s) in the waters that are responsible for the disparities observed. Therefore, the report recommended expanded analysis of the waters to include heavy metals and organic mineral parameters in future effort.

Declaration of conflict interests

There was absolutely no conflict of interest. Information sought for were meant for advancement of knowledge towards achieving increased fish production in the community where the study was carried out.

Ethical Approval code

Ethical approval was not sought prior to the commencement of the study since the fish "*Clarias gariepinus*" was studied within its natural ecosystem following established standard and there was no deliberate attempt to expose the fish to extraneous substance or environment inimical to its reproduction and development.

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